The Natural History of Inherited Retinal Dystrophy Due to Biallelic Mutations in the *RPE65* Gene

DANIEL C. CHUNG, METTE BERTELSEN, BIRGIT LORENZ, MARK E. PENNESI, BART P. LEROY, CHRISTIAN P. HAMEL, ERIC PIERCE, JULIANA SALLUM, MICHAEL LARSEN, KNUT STIEGER, MARKUS PREISING, RICHARD WELEBER, PAUL YANG, EMILY PLACE, EMILY LIU, GRACE SCHAEFER, JULIE DISTEFANO-PAPPAS, OKAN U. ELCI, SARAH MCCAGUE, JENNIFER A. WELLMAN, KATHERINE A. HIGH, AND KATHLEEN Z. REAPE

• PURPOSE: To delineate the natural history of visual parameters over time in individuals with biallelic *RPE65* mutation–associated inherited retinal dystrophy (IRD); describe the range of causative mutations; determine potential genotype/phenotype relationships; and describe the variety of clinical diagnoses.

• DESIGN: Global, multicenter, retrospective chart review.

• METHODS: Study Population: Seventy individuals with biallelic *RPE65* mutation–associated IRD. Procedures: Data were extracted from patient charts. Measurements: Visual acuity (VA), Goldmann visual field (GVF), optical coherence tomography, color vision testing, light sensitivity testing, and electroretinograms (retinal imaging and fundus photography were collected and analyzed when available).

• RESULTS: VA decreased with age in a nonlinear, positive-acceleration relationship ($P < .001$). GVF decreased with age ($P < .0001$ for both V4e and III4e), with faster GVF decrease for III4e stimulus vs V4e ($P = .0114$, left eye; $P = .0076$, right eye). On average, a 1-year increase in age decreased III4e GVF by ~25 sum total degrees in each eye while V4e GVF decreased by ~37 sum total degrees in each eye, although individual variability was observed. A total of 78 clinical diagnoses and 56 unique *RPE65* mutations were recorded, without discernible *RPE65* mutation genotype/phenotype relationships.

• CONCLUSIONS: The number of clinical diagnoses and lack of a consistent *RPE65* mutation–to–phenotype correlation underscore the need for genetic testing. Significant relationships between age and worsening VA and GVF highlight the progressive loss of functional retina over time. These data may have implications for optimal timing of treatment for IRD attributable to biallelic *RPE65* mutations. (Am J Ophthalmol 2019;199: 58–70. © 2018 Elsevier Inc. All rights reserved.)

INHERITED RETINAL DYSTROPHIES (IRDS) ARE A GROUP OF rare blinding conditions caused by mutations in any 1 of more than 250 genes.[1] The spectrum of disease attributable to biallelic mutations in 1 of those genes, *RPE65*, exhibits several common clinical findings, chiefly night blindness, progressive loss of visual fields, and loss of central vision.[2,3] However, age of onset, severity, rate of disease progression, and presenting clinical findings are variable, which leads to an assortment of clinical diagnoses being used for one and the same genotype, including, but not limited to, Leber congenital amaurosis (LCA), severe early childhood–onset retinal dystrophy (SECORD), early-onset severe retinal dystrophy (EOSRD), and retinitis pigmentosa (RP), which may all be grouped under the heading of *RPE65* mutation–associated IRD. Given that the U.S. Food and Drug Administration has approved a gene therapy for the treatment of patients with confirmed biallelic *RPE65* mutations, it is particularly important to obtain information about the natural course of genotype-specific disease entities for purposes of comparison with evolution after treatment. We have conducted such a study of biallelic *RPE65* mutation–associated IRD.

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From Research and Development, Spark Therapeutics, Inc, Philadelphia, Pennsylvania, USA (D.C.C., E.L., G.S., J.A.W., K.A.H., K.Z.R.); Department of Ophthalmology, Rigshospitalet, Glostrup, Denmark (M.B., M.L.); Department of Ophthalmology, Justus-Liebig-University Giessen, Giessen, Germany (B.L., K.S., M.P.); Ophthalmic Genetics Division, Casey Eye Institute, Oregon Health & Science University, Portland, Oregon, USA (M.E.P., R.W., P.Y.); Department of Ophthalmology, Ghent University Hospital and Ghent University, Ghent, Belgium (B.P.L.); Center for Cellular and Molecular Therapeutics (B.P.L.,S.M.) and Division of Ophthalmology (B.P.L.), Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; Maladies Sensorielles Génétiques, Montpellier, France (C.P.H.); Équipe Génétique et Thérapie des Cécités Rétiniennes et du Nerf Optique, INSERM U1051, Institute for Neurosciences of Montpellier, Montpellier, France (C.P.H.); Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, USA (E.Pierce. E.Place); Department of Ophthalmology, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil (J.S., J.D.-P., O.U.E.); and Biostatistics and Data Management Core, Westat, Philadelphia, Pennsylvania, USA (J.S., J.D.-P., O.U.E.).

Inquiries to Daniel C. Chung, Clinical Ophthalmic Lead, Spark Therapeutics, Inc, 3737 Market St, Philadelphia, PA 19104 USA; e-mail: daniel.chung@sparktx.com

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METHODS

RETROSPECTIVE CHART REVIEWS FROM 7 PARTICIPATING centers in 6 countries were conducted. Inclusion criteria were as follows: (1) male or female subject born between January 1, 1963 and December 31, 2010; (2) genetic diagnosis consistent with autosomal-recessive mutations in the *RPE65* gene; and (3) a minimum of 2 office visits/clinic encounters occurring prior to retinal surgery or enrollment in an interventional study for IRD. Subjects with other retinal disorders, ocular disorders that impact retinal function, or systemic disease associated with mutations in other retinal genes were excluded. All charts that met inclusion/exclusion criteria were included in the analysis.

Data collected and analyzed, when available and evaluable, included demographics, ocular history, medical/surgical history, genetic and clinical diagnosis, visual acuity (VA), Goldmann visual fields (GVF), optical coherence tomography (OCT), color vision, full-field light sensitivity threshold testing (FST), dark adaptation, electroretinography (ERG), retinal imaging, fundus photography, and short-wavelength (blue) light fundus autofluorescence (FAF). Protected health information was redacted prior to data collection. To avoid bias and improve consistency, criteria review and data entry for all charts were performed by the central principal investigator.

Based on the extent of data collected, the primary parameters analyzed were best-corrected VA (BCVA), GVF, and OCT. Because longitudinal data were not available for all subjects for all testing parameters, and the duration of follow-up varied, these primary assessments were analyzed using age as a proxy for time so that all available individual data points could be included in the analyses.

• **VISUAL ACUITY:** The method of VA assessment ranged from preferential looking Teller acuity cards for preverbal children to Allen and Snellen acuity charts for older subjects. For purposes of standardization, all VA assessments from the primary source were converted to decimals and then to logarithm of the minimum angle of resolution (logMAR) unit values using the following formula: LogMAR = −Log (Decimal Acuity).[4] VA results are presented in logMAR units, where smaller values indicate better VA. Off-chart VA measurements used adaptations of previously reported scales for assigning logMAR[4,5]; results are presented separately for each eye using the scales adapted from both Holladay[4] and Lange and associates.[5] The scales differ in that the estimates adapted from Holladay suggest a difference of a 1-log-unit step between, for example, counting fingers and hand motion perception, while use of the scale adapted from Lange reduces this difference to a 0.3-unit step. For the BCVA analyses, a 0.1 increase in logMAR corresponds to a 5-letter decrease on a standard Early Treatment Diabetic Retinopathy Study logMAR eye chart.

For the majority of available VA assessments, each eye was measured separately; bilateral VA assessments were more limited. For purposes of this analysis, VA results are presented for the right eye and left eye separately using standardized logMAR units. When available, BCVA was used, as this is the standard and customary method of collecting VA assessments; however, there were many subject charts where "BCVA" was not clearly specified. Therefore, it cannot necessarily be assumed that all VA measurements were BCVA, although it is likely that most were, since this is the standard method of testing. Regardless of whether BCVA was clearly stipulated, all available VA measurements were used in the analysis.

Data were collected from each subject over time at different ages, and longitudinal analyses for the effect of age on VA using both Holladay and Lange scales for off-chart measurements were performed. VA data obtained from subjects between the ages of birth to 3 years were excluded owing to the difficulty in obtaining reliable VA assessments in very young children.

• **VISUAL FIELDS:** Visual field (VF) assessments were collected over time at different ages using manual Goldmann kinetic perimetry. Cumulative VF calculations were conducted across 24 meridians for each stimulus tested in each eye separately. This outcome measure was based on the sum total meridian degrees (or sum total degrees) for each stimulus tested and represents a summation calculation based on the measure of degrees from central fixation to the point of the isopter intersection for each of the 24 meridians, including peripheral islands of retinal sensitivity when intersecting with a VF meridian and excluding areas of scotoma; higher sum total degrees indicate a greater area of functional, light-sensitive retina, corresponding to a greater field of vision for the subject. Using this approach, the maximal VF is approximately 1400 to 1800 (V4e) and 1200 to 1400 (III4e) sum total degrees in individuals without visual impairment.[6] Longitudinal analyses for the effect of age on VF by test stimulus type (III4e and V4e) were conducted.

• **SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY:** Longitudinal analyses for the effect of age on retinal thickness and outer nuclear layer (ONL) thickness, measured at the fovea, as determined by spectral-domain OCT were conducted. Thickness measurements were obtained by automated or manual segmentation using the nerve fiber layer and retinal pigment epithelium basement membrane as borders. For purposes of standardization, only data collected using Heidelberg technology were included in these analyses.

• **STATISTICAL METHODS:** Data were collected and summarized using descriptive measures, including means with standard deviations (SD) and medians with ranges and upper limits of the first and third quartiles for continuous

variables such as age, and frequencies and percentages for categorical variables such as sex. These represent the upper limits of the quartiles (ie, first quartile means greater than the first 25% of the data and the third quartile means greater than 75% of the data).

Cross-sectional analyses of longitudinal data were conducted to examine the association of age with VA, GVF by test stimulus type, and OCT findings. As it was difficult to determine a precise age of disease onset from the medical records, the subject's age at the time of initial testing was used as an approximation, using birth years. Mixed-effects linear/polynomial regression models implemented via maximum likelihood were applied separately to VA, VF, and OCT to determine whether age had an association with any of these parameters. Mixed-effects models account for correlations arising from the repeated measures and used all measurements obtained from each subject. Nonlinear effects of age (ie, quadratic term) were investigated and included in the models if they were significant. Sensitivity analyses were performed to examine the potential outliers and influential points. These measurements were assessed by visual examination of histograms and normal probability plots of residuals from each model. In the event that a subject had more than 1 measurement for the same eye on the same day, the average of the measurements was used for analyses. Stata 14.1 (Stata Corporation, College Station, Texas, USA) was used to conduct statistical analyses and the significance level was set at .05 for all tests.

Review and approval or waiver of the original study protocol was received from each affiliated study center's Institutional Review Board/Independent Ethics Committee in conformance with Good Clinical Practice: Consolidated Guideline as approved by the International Conference on Harmonisation and all applicable local and federal regulatory requirements were followed.

RESULTS

A TOTAL OF 102 POTENTIAL SUBJECTS WERE IDENTIFIED, and 32 were excluded, primarily owing to inadequate number of visits or birth date outside of the date range; thus, 70 charts met eligibility criteria and were included in the analysis. Although a minimum of 2 visits was an inclusion criterion, there was no specified minimum duration between visits. Of the 32 excluded subjects, most were out of the birth date range and/or had fewer than 2 visits. Many failed to meet more than 1 inclusion/exclusion criterion. One subject was diagnosed with a systemic disease associated with mutations in other retinal genes (protanopia [mutation in *OPN1LW* gene]). Fourteen subjects were out of the birth date range (1963-2013); of these, 5 also had fewer than 2 clinic visits. An additional 17 subjects had fewer than 2 clinic visits.

TABLE 1. Subject Demographics

Parameter/Category/Statistic	Eligible (n = 70)	Ineligible (n = 32)	Total (N = 102)
Age at initial visit (y)[a]			
Mean (SD)	15 (11.8)	15 (18.3)	15 (14.9)
Median (1st, 3rd quartile [upper limits])	9 (3, 18)	9 (3, 36)	9 (3, 24)
Min, max	1, 43	1, 61	1, 61
Sex, n (%)			
Female	42 (60.0)	21 (65.6)	63 (61.8)
Male	28 (40.0)	11 (34.4)	39 (38.2)
Race, n (%)			
White	47 (67.1)	23 (71.9)	70 (68.6)
Asian	2 (2.9)	1 (3.1)	3 (2.9)
Black or African American	14 (20.0)	3 (9.4)	17 (16.7)
Other	1 (1.4)	2 (6.3)	3 (2.9)
Unknown	6 (8.6)	3 (9.4)	9 (8.8)

[a]Age is approximate, since only birth years are available.

Owing to the retrospective nature of this study, not all charts contained the same information. Subject age ranged from 1 to 43 years (mean, 15 years) at the initial visit. Visits spanned up to 33 years (in 1 subject) with a mean duration of observation time of 7.3 years (median 4.5 years). Demographic information is shown in Table 1.

• **VISUAL ACUITY:** There was a total of 309 VA measurements for the left eye and 331 measurements for the right eye collected from 68 subjects. Each subject had a varying number of measurements (minimum, 1; maximum, 41). Not all measurements were obtained from all subjects at all visits. The age of subjects contributing VA measurements to the analysis ranged from 4 to 46 years. Age groups in 2-year increments through age 33 were created to summarize VA. Owing to the small number of measurements in subjects over age 34, the last age group included measurements from subjects 34-46 years of age.

The general pattern observed was that of marked impairment but fairly stable VA during the first decade of life, with gradual worsening beginning to occur around the ages of 15-20, and subsequent rapid acceleration of the rate of VA loss after the age of 20. The rate of vision loss was variable among subjects. Examination of the individual subject data suggests that there was no meaningful spontaneous recovery of VA in these subjects, although some showed an initial slight improvement in VA early in the disease course, which may be attributed to childhood maturation and development (reading skills, attention span, etc), resulting in better testing performance.

The mean VA in the youngest age group was 0.8 logMAR (where 0 logMAR is equivalent to 20/20 on the Snellen acuity scale; 0.8 logMAR is approximately equivalent to a Snellen value of 20/126). Around the age of 16

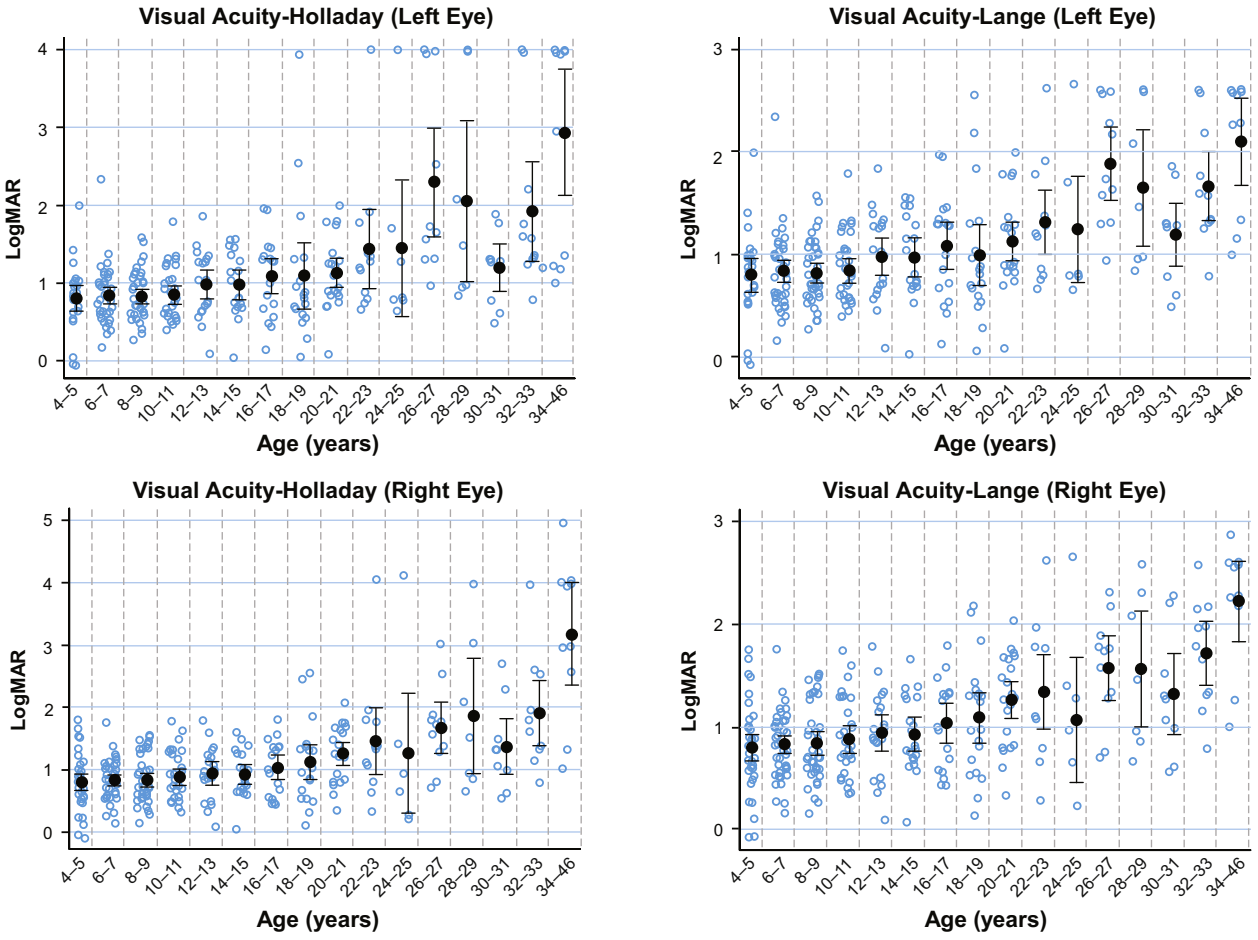

FIGURE 1. Visual acuity by age group (left and right eyes).

years, there appeared to be a greater change in the median logMAR, where it began to exceed 1.0 (equivalent to 20/200, or legally blind by United States standards).[7] VA subsequently worsened and the logMAR exceeded 2.0 (equivalent to 20/2000) in the oldest subject group (34-46 years), regardless of whether the Holladay or Lange scale was used to estimate logMAR for off-chart assessments.

Figure 1 shows the average VA with 95% confidence intervals (CI) and the measurements at each age group for the left and right eyes, using Holladay and Lange methods for off-chart estimates. The modeled quadratic trends of age on VA were statistically significant ($P < .001$) for each eye, consistent with the pattern of observed means appearing in Figure 1. VA worsened with age, but at differing rates, depending on age. The worsening of VA appeared greater in older subjects. The mean VA for all subjects (all VA test results across all age groupings) falls into the "low vision" category (0.6 logMAR, equivalent to 20/80).[8]

• **VISUAL FIELDS:** GVF (in sum total degrees) was calculated for each eye separately in 42 subjects (inclusive of subjects tested with III4e, V4e, and other test stimuli, as well as subjects with nonrepeated measurements). For this analysis, 27 subject charts contributed repeated III4e and/or V4e measurements, with 161 GVF measurements recorded for the left eye and 160 measurements for the right eye. There was a similar number of III4e and V4e measurements for each eye. The age range of contributing subjects was 6-34 years and, similar to the VA analysis, age groups in 2-year increments were created to summarize VFs from 6 to 21 years of age. Owing to the small number of measurements in subjects 22-37 years of age, the last 2 age groups included measurements from subjects 22-27 and 28-34 years of age.

To determine the association of age with VF, mixed-effects linear regression models used sum total degrees as the outcome and linear age, random intercept, and age by test stimulus type (III4e vs V4e) interaction. There was a negative relationship between age and VF, with GVF decreasing for both the III4e and V4e test stimuli as age increased. The effect of age on VF demonstrated a faster decline in sum total degrees with the III4e stimulus vs the slower decline seen with the V4e stimulus. There was

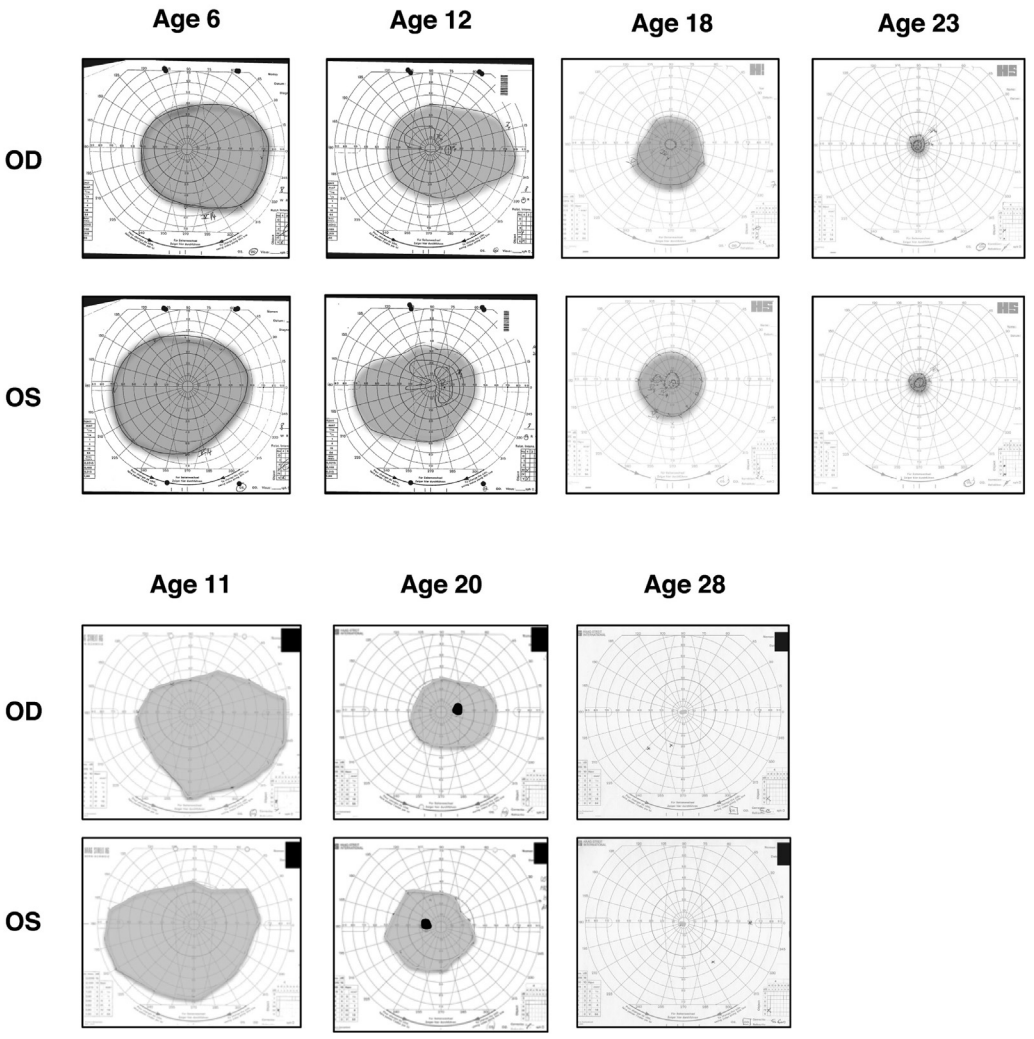

FIGURE 2. Goldmann kinetic visual field (V4e) over time in 2 representative subjects. Progressive visual field loss measured with the target in 2 subjects.

variability in sum total degrees, and SDs were wide. In the youngest age group, mean GVFs were 865 and 862 degrees for the III4e stimulus for the left eye and right eye, respectively, and 825 and 821 degrees for the V4e stimulus, respectively. The III4e fields decreased to a mean range of 85-90 sum total degrees around the age of 20 and older (an approximate 90% reduction from the mean values in the youngest subjects). Since the V4e target is much larger, it is less discriminatory, and VFs measured using this stimulus may be preserved for a longer period of time. However, it appears that once VFs began to constrict with V4e, the reduction occurred at a faster rate than that observed using the III4e stimulus. In the older subjects (aged 28-34 years), the mean V4e fields were reduced approximately 70% from the youngest subjects, to an average of approximately 235 sum total degrees. Two representative GVF maps are shown in Figure 2.

VF decreased by age for both test stimulus types (III4e and V4e) and for both eyes ($P < .0001$). Results from the mixed-effects models showed a statistically significant interaction effect of age by test stimulus type for the left eye ($P = .0114$) and right eye ($P = .0076$). The decrease in VF was faster for V4e stimulus compared with III4e stimulus with increasing age. On average in this cohort, a 1-year increase in age decreased the III4e GVF by approximately 25 sum total degrees in each eye while the V4e GVF decreased by approximately 37 sum total degrees in each eye, as seen in Figure 3.

• **SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY:** All retinal thickness and ONL thickness values were measured at the fovea using spectral-domain OCT. Data were analyzed for the left eye and right eye separately. A total of 54 retinal thickness and 53 ONL measurements for the left eye and 53 retinal thickness and 52 ONL measurements for the right eye were collected from 32 subjects. The age of subjects ranged from 6 to 38 years, with the number of measurements obtained from each subject

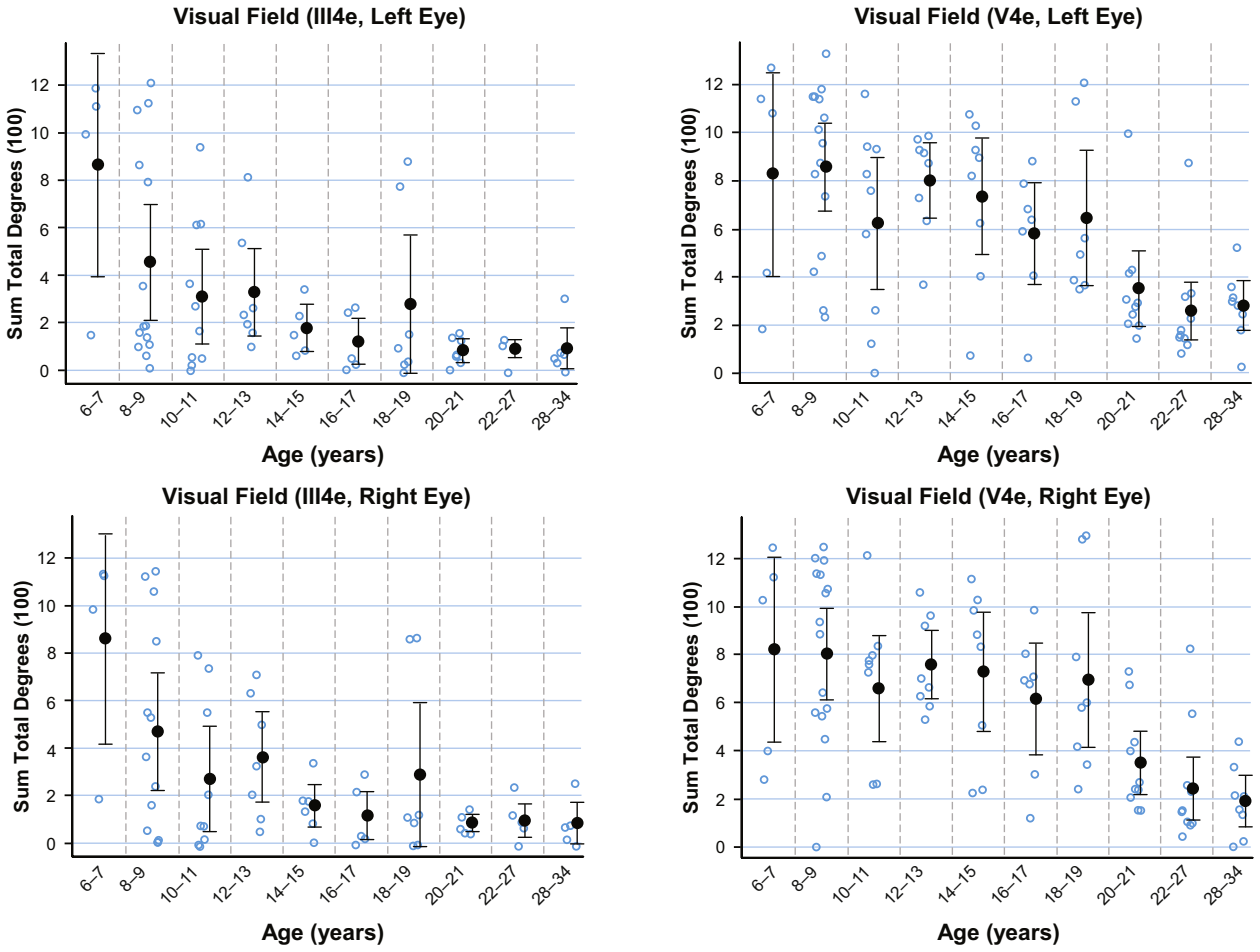

FIGURE 3. Mean Goldmann visual fields by age and stimulus type (left and right eyes). The lower limit of normal for the sum total degrees of Goldmann Visual Field III4e is 1200 and for Goldmann Visual Field V4e is 1400.

ranging from 1 to 3. Eleven subjects had only 1 measurement of retinal thickness and 10 subjects had only a single ONL thickness measurement. The median period from the first to last OCT recorded for the remaining subjects was 1.9 years (range, 0-4.8 years) for both retinal and ONL thickness.

Mixed-effects linear regression models with random intercept were applied to retinal and ONL thickness. Results from the mixed-effects linear regression models showed no statistically significant effect of age on retinal thickness for the left eye ($P = .4935$) or right eye ($P = .3158$), or on the ONL thickness for the left eye ($P = .4811$) or right eye ($P = .1818$).

• **SECONDARY PARAMETERS:** *Clinical Diagnosis*. A total of 78 clinical diagnoses were reported in the charts of the 70 subjects, with some subjects having more than 1 diagnosis at the first reported visit where a clinical diagnosis was available. In addition, subjects may have received 1 clinical diagnosis early, which was subsequently changed over time as more information (usually genetic testing results) became available. To be eligible for this study, a subject chart had to have a confirmed genetic diagnosis consistent with mutations in the *RPE65* gene. Among the 78 clinical diagnoses at the time of the first reported visit, 37 (47.4%) were LCA, 6 (7.7%) were RP, 5 (6.4%) were tapetal retinal dystrophy, 4 (5.1%) were SECORD, and 2 (2.6%) were EOSRD. Three subjects each had a clinical diagnosis of "low vision" or "tapetal retinal dystrophy, Leber type," while 2 subjects had a clinical diagnosis of "cone-rod dystrophy." There were 16 subjects who had other unique clinical diagnoses. In total, there were 24 distinct clinical diagnoses assigned to this study population at the time of the initial visit. There were 31 subjects who had more than 1 clinical diagnosis over the course of their visits, and of these 31, the average number of clinical diagnoses was 3, with a minimum of 2 and maximum of 7. There were 9 subjects who received a diagnosis of both LCA and RP over the course of their visits. These diagnoses are shown in Figure 4.

The age at clinical diagnosis was obtained from the 70 subject charts. Sixty-seven (95.7%) subjects were 18 years

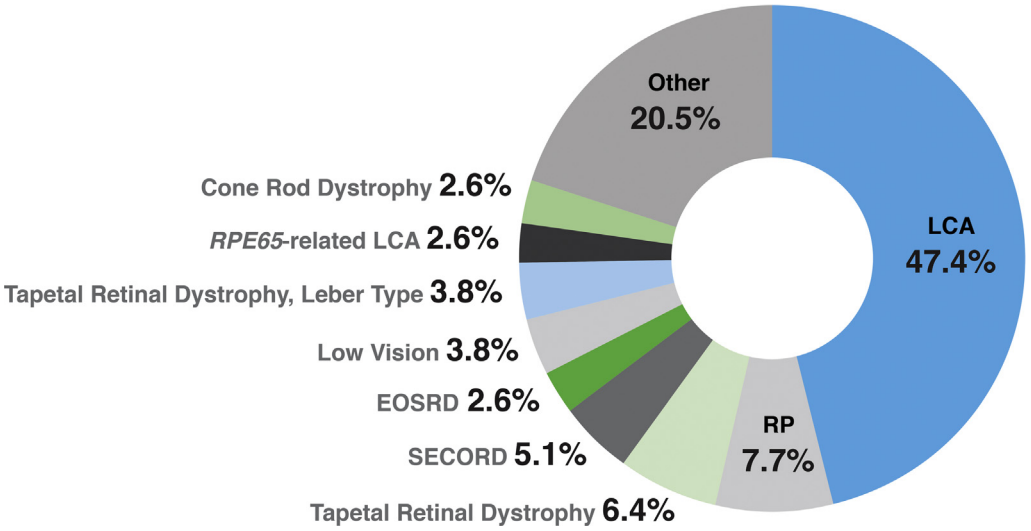

FIGURE 4. Clinical diagnosis at first visit. Numbers add to more than 100% because some patients had more than 1 diagnosis. EOSRD = early-onset severe retinal dystrophy; LCA = Leber congenital amaurosis; RP = retinitis pigmentosa; SECORD = severe early childhood–onset retinal dystrophy.

of age or younger at the time of the first recorded clinical diagnosis or the onset of symptoms noted in the ocular history. The majority of clinical diagnoses were made in subjects aged 1-5 years (42.9% [n = 30]), followed by subjects 18 years of age and over (25.7% [n = 18]), subjects aged 6-10 years (14.3% [n = 10]), subjects aged 11-17 years (10% [n = 7]), and subjects less than 1 year of age (7.1% [n = 5]). The remaining 3 subject charts contained no documentation regarding the age of onset of symptoms or prior care (including verbal assessments) by other retinal specialists.

Genetic Diagnosis. All eligible charts had confirmation of biallelic *RPE65* mutations, unequivocally confirming a diagnosis of autosomal-recessive *RPE65*-related IRD. Mutations were converted to a standard format according to Human Genome Variation Society guidelines. A total of 56 unique *RPE65* mutations were observed in this study population, with 27 subjects (38.6%; 9 homozygous) having at least 1 mutation known from the literature to be associated with LCA, 37 subjects (52.9%; 7 homozygous) having at least 1 mutation associated with SECORD or RP, and 18 subjects (25.7%; 9 homozygous) having other mutations not previously reported in the context of any of these clinical diagnoses. A summary of all *RPE65* mutations is provided in Table 2.

Electroretinography Assessments. Among the 70 subjects, there were 98 ERG assessments performed in 60 subjects, with some subjects having more than 1 assessment. There were no normal results for any of the 60 subjects who had evaluations. For dark-adapted scotopic (rod) ERGs, 16 (16.3%) showed some residual activity and 77 (78.6%) were extinguished. For light-adapted photopic (cone) ERGs, almost twice the number of assessments (n = 31; 31.6%) demonstrated some minimal activity compared with scotopic ERGs, while 61.2% (n = 60) were extinguished. The mean age (SD) for those with residual scotopic activity (n = 16 subjects) was 10.5 (9.5) years while the mean age (SD) for those with residual photopic activity (n = 31 subjects) was 9.8 (8.1) years.

Other Assessments. A total of 70 color vision testing results were available from 31 subjects, although the specific testing methods varied. In this study cohort, Ishihara plates and the Farnsworth D-15 assessment were the most frequently used color vision tests. Of the 70 color testing results, a total of 60 (85.7%) showed some abnormality in color vision, 4 (5.7%) of the results showed normal color vision, and 6 (8.6%) yielded inconclusive testing results. The most frequently occurring anomaly in color vision was disturbances in the tritan (blue/yellow) axis, accounting for one third of all abnormalities.

A total of 64 subject charts reported refractive error data; 8 of these subjects had cycloplegic refraction only, and were based on the findings at the first visit with a manifest or cycloplegic refraction. Refractive errors were converted to spherical equivalents. The majority of refractive errors were myopic (54.7% [n = 35]) and hypermetropic (39% [n = 25]); 4 individuals (6.3%) showed emmetropia. Within the myopia group, 54.3% were considered low (<-3.00 diopters [D] [n = 19]), 20% moderate (-3.00 to -6.00 D [n = 7]), and 25.7% high (>-6.00 D [n = 9]). Within the hypermetropia group, 68% were considered low (<2.00 D [n = 17]), 24% moderate (2.25 to 5.00 D [n = 6]), and 8% high (>5.00 D [n = 2]). There was 1 (1.6%) instance of anisometropia. As the refractive error data were collected at the first visit in which manifest or cycloplegic refraction was performed, variability can be

TABLE 2. *RPE65* Mutations in the Subject Population

Number of Subjects	Mutation 1	Mutation 2
5	c.329A>G, p.Asp110Gly	(homozygous)
2	c.138delG, p.Pro47Glnfs47	(homozygous)
2	c.304G>T, p.Glu102	(homozygous)
2	c.271C>T, p.Arg91Trp	c.304G>T, Glu102
2	c.271C>T, p.Arg91Trp	(homozygous)
2[a]	p.Arg118Ser	p.Val443Ala
2	c.700C>T, Arg234	c.1120delA, p.Ile374Leufs
1	c.131G>, p.Arg44Gln	(homozygous)
1	c.131G>, p.Arg44Gln	(homozygous)
1	c.11+5G>A	c.310G>A, p.Gly104Ser
1	c.11+5G>A	c.1590delC, p.Phe530Leufs
1	c.11+5G>A	c.1370C>A, p.Thr457Asn
1	c.271C>T, p.Arg91Trp	c.1102T>C, p.Tyr368His
1	c.271C>T, p.Arg91Trp	c.1102T>C, p.Tyr368His
1	c.11+5G>A	c.144_145insT, p.Leu49Serfs
1	c.11+5G>A	c.1102T>C, p.Tyr368His
1	c.651delA, Glu217Glufs	c.1580A>C, p.His527Pro
1	c.74C>T, p.Pro25Leu	(homozygous)
1	c.11+5G>A	c.725+2T>A
1	c.74C>T, p.Pro25Leu	c.235T>C, p.Tyr79His
1	c.271C>T, p.Arg91Trp	c.304G>A, p.Glu102Lys
1[a]	p.Asn135Lys	c.209T>C, p.Phe70Ser
1	c.1022T>C, p.Leu341Ser	(homozygous)
1	c.495_495+1insG	c.1374G>A, p.Trp458
1	c.715T>A, p.Tyr239Asp	c.1102T>C, p.Tyr368His
1	c.1102T>C, p.Tyr368His	c.130C>T, p.Arg44
1	c.329A>G, p.Asp110Gly	(homozygous)
1	c.11+5G>A	c.1088C>G, p.Pro363Arg
1	c.11+5G>A	c.1102T>C, p.Tyr368His
1	c.329A>G, p.Asp110Gly	(homozygous)
1	c.130C>T, p.Arg44	c.1543C>T, p.Arg515Trp
1	c.130C>T, p.Arg44	c.1543C>T, p.Arg515Trp
1	c.94G>T, p.Gly32Cys	c.130C>T, p.Arg44
1	c.1328T>C, p.Val443Ala	c.1102T>C, p.Tyr368His
1	c.130C>T, p.Arg44	c.495_495+1insG
1[a]	c.11+2T>_?	c.74C>T, p.Pro25Leu
1	c.370C>T, p.Arg124	c.952T>A, p.Tyr318Asn
1	c.545A>G, p.His182Arg	(homozygous)
1[a]	p.Lys294ins___?	c.1244C>T, p.Ala415Val
1	c.429C>G, p.Tyr144	c.1351A>T, p.Asn451Tyr
1	c.406G>T, p.Val136Phe	(homozygous)
1	c.11+5G>A	c.1543C>T, p.Arg515Trp
1[a]	c.1336dupA?	(homozygous)
1	c.208T>G, c.1102T>C	c.208T>G, c.1102T>C
1	c.289dupA, p.Arg97Lysfs	(homozygous)
1	p.Trp460Cys	c.1451G>A, p.Gly484Asp
1	c.370C>T, p.Arg124	c.272G>A, p.Arg91Gln
1	c.1102T>C, p.Tyr368His	c.1292A>G, p.Tyr431Cys
1	c.235T>C, p.Tyr79His	c.1445A>G, p.Asp482Gly
1	c.11+5G>A	c.1022T>C, p.Leu341Ser
1	c.247T>C, p.Phe83Leu	(homozygous)
1	c.272G>A, p.Arg91Gln	c.137G>A, p.Gly46Glu
1	c.61G>T, p.Glu21	c.1022T>C, p.Leu341Ser
1	c.272G>A, p.Arg91Gln	(homozygous)

Continued on next page

TABLE 2. *RPE65* Mutations in the Subject Population (*Continued*)

Number of Subjects	Mutation 1	Mutation 2
1	c.1336dupA, p.Arg446Lysfs	(homozygous)
1	c.272G>A, Arg91Gln	c.1022T>C, p.Leu341Ser
1	c.95-2A>T	(homozygous)
1	c.95-2A>T	(homozygous)
1	c.843_858del+7	(homozygous)
1	c.444G>C, p.Glu148Asp	c.1451G>A, p.Gly484Asp

[a]Genotyping information available was not sufficient to convert to Human Genome Variation Society guidelines.

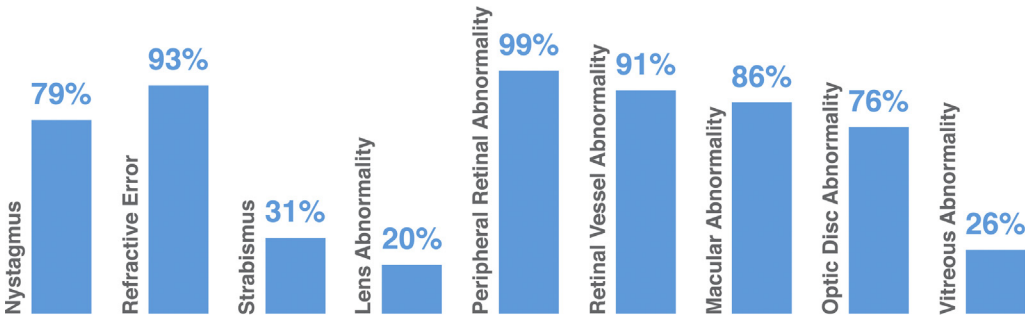

FIGURE 5. Ocular findings in patients with biallelic *RPE65* mutation–associated inherited retinal disease.

large owing to the different developmental age of the subject. A subset of 18 subjects (aged 10-15) was analyzed, of which 66.7% were myopic and 33.3% were hypermetropic.

Ocular findings are summarized in Figure 5. Almost 80% (n = 55) of subjects had at least 1 visit where the presence of nystagmus in at least 1 eye was noted, although not every chart had this information. All subjects with nystagmus were affected bilaterally. In 12 (17.1%) subjects, nystagmus was either not evaluated or not recorded; only 3 subjects (4.3%) were noted specifically as not having nystagmus. Of those with nystagmus, it was present at the first recorded clinic visit in 40 (72.7%) subjects, with an average age of approximately 19 years at the time of the first report of nystagmus. There were 30 subjects (43%) who had nystagmus noted during at least 1 visit who did not have a clinical diagnosis of LCA at any visit. Over the course of the study visits, 3 subjects who had previously had nystagmus in both eyes had nystagmus recorded as absent at the final visit, and another subject with prior nystagmus showed absence of nystagmus in just the right eye at the final visit.

Ocular misalignment was recorded in 22 (31.4%) subjects. Of those with misalignment, exotropia was reported in 12 (54.5%) and esotropia was reported in 10 (45.5%). Ocular alignment was assessed from the first visit at which it was reported.

All subjects in this cohort had their lenses examined bilaterally, and abnormalities were noted as present or absent. A total of 14 (20.0%) subjects had cataracts or other lens opacities/abnormalities in at least 1 eye; of those 14 subjects, 11 (78.5%) had bilateral lens abnormalities; 2 subjects had lens abnormalities in only the right eye and 1 subject had a lens abnormality in only the left eye. The types of cataracts included posterior subcapsular (43% [n = 6]), cortical (29% [n = 4]), nuclear sclerotic (7% [n = 1]), and unspecified (36% [n = 5]). Two subjects were reported to have multiple lens opacities. The mean age of subjects at the time the first lens abnormality was approximately 26 years. All subjects were phakic at the time of their first recorded visit.

Peripheral retinal abnormalities, comprising mainly outer retinal atrophy and pigmentary changes, were reported in 69 subjects (98.6%), and retinal vascular attenuation was noted in 64 subjects (91.4%). Macular abnormalities, including abnormal foveal reflex and atrophic changes, were reported in 60 subjects (85.7%), and optic disc pallor and related abnormalities, such as waxy optic disk, were reported in 53 subjects (75.7%). Most subjects had findings in more than 1 posterior segment component (vitreous, optic nerve, macula, vessels, and periphery), with 36 (51.4%) having 4 abnormal components and 14 (20%) having abnormalities in all 5 components. The age at the first documentation of any posterior segment abnormality at the study center was approximately 12-13 years and was dependent upon the age of presentation to the

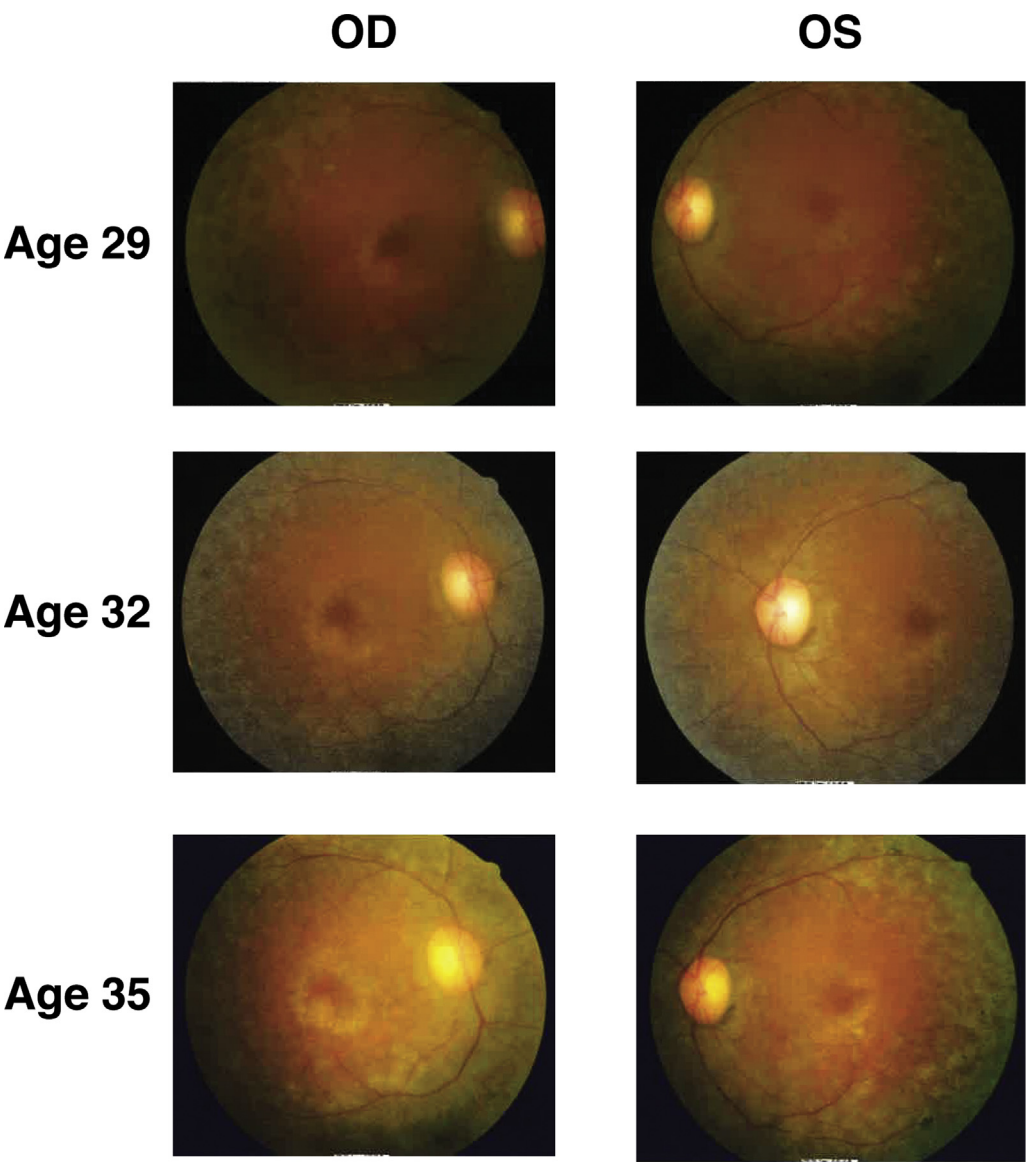

FIGURE 6. Progressive fundus changes in an individual subject occurring over 6 years. This is representative of the degree and progression of fundus changes for patients with biallelic *RPE65* mutation–associated inherited retinal disease associated with biallelic *RPE65* mutations.

specialist center. This may not be reflective of the true age of onset.

The lack of standardization and variation in image quality precluded formal analysis of fundus photography. However, in general, fundus abnormalities (pigmentary changes, retinal atrophy, vessel attenuation, optic nerve pallor), while variable, appeared to be more pronounced at older ages, as seen in Figure 6. FAF testing was reported in 26 subjects from 4 study sites. Twenty-three (88.5%) of these subjects showed severely diminished or absent autofluorescence and 3 (11.5%) showed small focal areas of FAF in the fovea/posterior pole. Only 3 subject charts had recorded FST testing results, precluding any formal analysis of this parameter.

Ocular history was not collected in a standardized fashion and reflects only information available from the subject charts. In this cohort, almost three quarters of subjects (74.3%) reported some orientation and/or mobility issues, while 80% reported at least some use of low vision aids, ranging from glasses to magnifiers to the use of Braille. More than half of the subject charts (54.3%) included comments related to poor night vision, and 88.6% reported photophobia. The mean age at which poor night vision was first reported was 12.10 years (range, 2-43 years); although the upper range of the first report of poor night vision is 43, onset may have been much earlier, as onset of night vision loss at age 43 would be highly improbable. Photophobia was reported at an older mean

age of 17.27 years (range, 4-43 years). In general, there was a clear progression of worsening vision, decreasing VFs, and increasing reliance on visual aids, providing evidence of the decline in the ability to function independently as retinal degeneration continued and visual function became increasingly compromised.

DISCUSSION

TWO PREVIOUS STUDIES HAVE REPORTED THE NATURAL history of LCA over time, although these patients did not have genotyping and almost certainly had mutations in a variety of genes.[9,10] In a cohort study by Heher and associates,[9] the VA of 22 patients with an initial diagnosis of LCA, evaluated on 2 or more occasions over 1-13.5 years (mean, 5 years), remained stable despite progressive retinal pigmentary changes. Paunescu and associates[11] reported the longitudinal findings of 7 patients with *RPE65* mutations and a clinical diagnosis of EOSRD. In addition, these data were combined with those of other case reports in the literature, for a total of 78 patients in 49 families with EOSRD or LCA associated with mutations in *RPE65*.[11] A trend was noted toward decreasing VA with increasing age. GVF data were only available for 16 patients and testing was performed at least twice in only 8 patients in whom progressive narrowing of VF was observed. Funduscopic appearance was available for 34 patients who had normal or minimally abnormal fundus findings in the first decade, with progressive macular and/or peripheral changes beginning in the second decade. Therefore, our study is the largest single cohort of patients with biallelic *RPE65*-associated IRD evaluated over time to date. All study centers were tertiary care retinal referral centers, and thus the charts did not always contain complete information from evaluations prior to referral. Furthermore, the number of examinations and specific data documented varied among subjects. These common limitations of retrospective chart reviews are important to consider as one evaluates the findings of the study. However, unlike previous natural history reports, all data were analyzed in the same manner and pooled to provide a complete and statistically sound analysis. In addition, ours was the first study to provide information on *RPE65* mutations in study patients. Therefore, we feel that our data provide powerful aggregated information regarding the natural history of *RPE65* mutation–associated IRD, using age as a surrogate for time. The primary parameters analyzed (VA, VF, and OCT) were collected at multiple visits over time, allowing for a robust analysis of these data.

Prior reports[2,3] have illustrated some associations of genotype to disease onset and progression, and that certain clinical findings, such as absent FAF,[12] may be attributed to mutations in the *RPE65* gene. It has been noted that FAF is absent in patients with autosomal-recessive *RPE65* mutation–associated IRDs. Some of the FAF data collected in this study may have been obtained with increased sensitivity of the capture system in order to acquire an FAF image, thus artificially enhancing FAF. However, evaluation of specific mutations in the *RPE65* gene in this study showed a lack of clear correlation between molecular diagnosis (ie, mutation) and a descriptive diagnostic label based on onset, severity, and rate of progression (ie, clinical diagnosis). It should be noted that *RPE65* mutation–associated IRDs may pose special challenges for genotype/phenotype correlations, as the phenotype resulting from biallelic *RPE65* mutations appeared to be relatively uniform and independent of mutation class, suggesting that most missense mutations result in loss of function. Since a number of different combinations of *RPE65* mutations can show a severe phenotype, some missense mutations may result in true null alleles whereas others may reduce the effectiveness of the protein product by other means.[13] Alternatively, or in addition, variability of disease severity may result from modifier genes impacting *RPE65* mutation–associated cell biology/physiology. Owing to the retrospective multicenter nature of this study and the variation of usage of descriptive clinical terms, a deeper formal analysis of genotype/phenotype associations was not attempted.

While some individuals had normal or near-normal VA at young ages, in this cohort, on average, cross-sectional data showed that VA was markedly impaired and fairly stable during the first decade of life, began to decrease around the ages of 15-20, and decreased more rapidly after the age of 20. This is consistent with the Paunescu longitudinal and cross-sectional natural history data,[11] which showed a notable deterioration in both VA and VF during the second and third decades of life.[8] As might be expected with the known heterogeneity of this condition, this pattern of vision loss was observed in the mean and median results for the entire study group, but not always at the individual subject level. Some individuals maintained the same VA for many years and then had a pronounced deterioration in a relatively short period of time, while others had more gradual loss of VA over the years.

Based on classifications in the International Council of Ophthalmology (ICO) 2002 report on the aspects and ranges of vision loss, the mean VA for the "low vision" category ranges from ≥0.6 to ≤1.3 logMAR, equivalent to Snellen VA of ≥20/80 or ≤20/400 in both eyes.[8] In the United States, this degree of vision loss qualifies individuals for special education benefits.[8] VA appears to deteriorate around the age of 16 years, when the mean logMAR begins to exceed 1.0 (equivalent to 20/200 Snellen VA); this is the ICO's threshold for severe visual impairment[8] and the threshold for "legal blindness" in the United States.[7] By the age of 18 years, more than half of this cohort are legally blind, as defined by the VA of the better eye. In the present study, VA continued to worsen, with a more rapid acceleration, and the mean logMAR exceeded 2.0 (equivalent to 20/2000) in the oldest subject group (aged 34-46

years), regardless of whether the scale adapted from Holladay or Lange was used for off-chart assessments.

Furthermore, the rates of change in VA were variable, depending on the size of the age groupings and the number of data points within each group. Therefore, it was difficult to calculate a reliable time-dependent rate of change in VA for the study cohort, given the nonlinearity of the 2 variables. Any overall rate of change would tend to be an overestimate for those who were in a more stable phase and could be an underestimate for those who were in an accelerated phase of deterioration.

Similar to the observed changes in VA, GVF decreased with age, although there were fewer individual subjects with evaluable longitudinal VF assessments. There was a fair amount of variability in the median and mean measures for both III4e and V4e test stimuli. As expected, the V4e field was larger for all age groups and was preserved for a longer period of time than the III4e field. However, even though the onset of field constriction for the V4e isopter was later in the disease course than the III4e isopter, the actual rate of change for the V4e field was approximately 50% faster than the III4e field. VF decline appeared to be more linear and gradual than VA loss, occurring over several decades. Regardless, both stimuli test types (III4e and V4e) showed significant decreases with age in this study population. With either stimulus, on average, subjects had two thirds or less of the total normal VF beginning in the earliest age category in which fields were evaluated (6-7 years). The ICO's 2002 report on the aspects and ranges of vision loss suggests that 50% of normal constitutes moderate impairment, whereas the American Medical Association Guides (2001) classify 30%-49% impairment of the field as moderate vision loss and 50%-69% as severe vision loss.[8,14] Around age 14 to 15 years, on average, the degree of impairment as measured by GVF III4e for the subjects in this natural history study began to exceed the ICO's threshold for severe visual impairment[8] and the "legal blindness" criteria in the United States (one-sixth normal VF, or approximately 235 sum total degrees).[7]

Compared with VA and VF assessments, very few subjects had multiple OCT measurements over long periods of time, making it difficult to determine the natural history of changes in the retinal layers in this population. With this limitation in mind, no statistically significant age effect on retinal or ONL thickness was found. With retinal degeneration and loss of cells, the retinal thickness and ONL thickness would be expected to decline with age. This pattern was not observed in this study cohort, most likely owing to the limited quantity of OCT data available for analysis and the relatively short duration of follow-up (median of 1.9 years between first and last result).

Consistent with diagnostic criteria for retinal disease attributable to biallelic *RPE65* mutations, ERGs were observed to be extremely compromised and of the rod-cone dystrophy type, or undetectable early in the natural course of this condition, with all subjects in the study cohort demonstrating severe abnormalities. Slight variations may be attributable to time of onset, as those with later onset may tend to have residual photopic responses.

Color vision deficiencies were common, with one third of the abnormalities affecting the tritan (blue-yellow) axis. This is consistent with a prior report of a significant deficit in blue cones in this patient population.[15] The abnormalities in color vision are consistent with cone involvement, which would be expected based on the ERG and VA data, and perhaps attributable to more advanced retinal degeneration in these individuals.

Ocular history data, consisting primarily of observational notations, show disease progression as it impacts daily functioning. According to the charts assessed, subjects had increasing dependence on mobility and visual aids with advancing age, a trend more readily apparent in those subjects with long-term longitudinal ocular history data. Low vision necessitated the use of vision aids in 80% of the population, while 74% experienced mobility issues as documented at the first visit. Nyctalopia, considered a clinical hallmark of this condition, was reported in the majority of subjects, as was photophobia. Specific rates should be interpreted with caution, as they are based solely on historical notes recorded in the chart, and the sources (patient and/or caregiver) and methods of collection (directly solicited or volunteered) are unknown and possibly incomplete. Paunescu and associates[11] reported the absence of photophobia in childhood, with development in adulthood, in subjects with a clinical diagnosis of EOSRD associated with *RPE65* mutations.[8] The observations from the current study may be reflective of the age of the subjects in this cohort at the time of the initial visit, the duration of follow-up, and/or the limitations of the study design.

The retrospective nature of this study has limitations, primarily the lack of standardization of the data collected and the evolution of imaging technology during the period of observation. However, the study design is appropriate for this patient population, given the rarity and rate of progression of the disease. The number and variety of clinical diagnoses found within *RPE65*-LCA, the limited information about phenotype correlations within the clinical spectrum of *RPE65*-LCA, and the even wider variation across all the different types of LCA underscore the need for genetic testing. Additionally, interpretation of the retinal layer thickness measurements may be limited owing to the short longitudinal time span between OCT measurements. Limitations in available data may be due to both relatively late introduction of spectral-domain OCT to the clinic and the reduced quality of OCTs in subjects with nystagmus.

Findings from this natural history study demonstrate a clear age-related decline in VA and VF in individuals with confirmed biallelic *RPE65*-associated IRD, with no evidence of spontaneous sustained improvement in either of these parameters. The prevalence of abnormalities in ERGs, color vision, and other ophthalmologic findings

within our study population is, for the most part, in keeping with the known clinical presentation. Structural abnormalities on ophthalmologic examination (lens, macula, retinal vessels, and periphery) also appear to increase with the age of the subject, consistent with progressive retinal degeneration. Despite variability in VA and VF decline, the statistically significant relationships between age and worsening VA and diminishing VF have implications for optimal timing of treatment for *RPE65* mutation–associated IRD.

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In Memoriam: Professor Christian Hamel died on August 15, 2017, at the age of 62 years. He was a pioneer in the field of ophthalmic genetics, with a concentration in inherited retinal disorders, and was the head of the Department of Genetic Sensory Diseases at the Montpellier University Hospital and Director of the Neuroscience Institute of Montpellier, Montpellier, France. Prof. Hamel is credited as one of the discoverers of the *RPE65* gene, a cause of a rare form of genetic blindness, which laid the groundwork for the understanding of this gene and the subsequent gene therapy studies that have led to a potential gene-based treatment for these patients. Prof. Hamel was a consummate scientist, clinician, and champion for those with inherited retinal disease and was dedicated to their care and to the discovery of potential treatments.

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